

**67.** A multiplex lateral flow assay comprising:

- i) a lateral flow cassette with two or three lanes for determining the concentration of analytes in a biological sample obtained from a patient wherein said analytes comprising Flt3-L and Rap1-GTP, and optionally Rac1-GTP;
  - ii) a sample and buffer zone in each lane where a sample is to be introduced onto the assay;
  - iii) a detector or primary capture zone in each lane where FLT3L or a Rap1-GTP analytes become bound to detector reagents wherein said detector reagents comprise antibodies or proteins which are analyte specific and wherein each detector reagent is conjugated to a reporter and each of said detector reagents is capable of flowing through said lane of said assay after binding said analyte;
- an analyte capture zone comprising analyte capture lines for FLT3L and Rap1-GTP each of which capture lines comprises a support which is conjugated to a capture reagent comprising a capture antibody or protein which is specific for binding said FLT3L detector reagent said Rap1-GTP detector reagent and wherein said FLT3L detector reagent or said Rap1-GTP detector reagent becomes concentrated at said capture line after binding to said capture reagent; and
- vi) a terminal wick zone which allows wicking of solution which has flowed through the assay to its terminal zone.

**68.** The multiplex assay according to claim **67** further comprising in a separate lane of said assay or in the same lane that is used to measure the concentration of Rap1-GTP analyte in said biological sample,

- i) a detector reagent in said detector or primary capture zone specific for Rac1-GTP comprising antibodies or proteins which are Rac1-GTP specific and wherein said detector reagent is conjugated to a reporter and is capable of flowing through said lane of said assay after binding said analyte; and
- ii) a capture reagent comprising a capture antibody or protein which is specific for binding said Rac1-GTP bound detector reagent wherein said capture reagent is bound to a support at a capture line in said analyte capture zone of said lane and said Rac1-GTP bound detector becomes concentrated after binding to said capture reagent at said capture line.

**69.** The assay according to claim **68** wherein said detector reagent and capture reagent for Rac1-GTP is in the same lane as the detector reagent and capture reagent for Rap1-GTP.

**70.** The assay according to claim **68** wherein said detector reagent and capture reagent for Rac1-GTP is in a separate lane from the detector reagent and capture reagent for Rap1-GTP.

**71.** The assay of any of claims **67-70** wherein said reporter is a color bead, a quantum dot or colloidal gold.

**72.** The assay of any of claims **67-71** wherein said reporter is a color bead or quantum dot.

**73.** The assay of any of claims **67-71** further comprising non-specific antibodies or non-specific proteins for enhancing the accuracy of the immunoassay.

**74.** The assay of any of claims **67-73** wherein said Flt3-L detector reagent is an anti-Flt3-L antibody.

**75.** The assay of claim **74** wherein said antibody is a monoclonal antibody.

**76.** The assay according to any of claims **67-75** wherein said Rap1-GTP detector reagent is an effector protein comprising RalGDS-RBD.

**77.** The assay according to claim **76** wherein said effector protein is GST RalGDS-RBD fusion protein.

**78.** The assay according to any of claims **67-77** wherein said reporter is a color bead or quantum dot.

**79.** The assay according to any of claims **67-78** wherein said reporter is a color bead.

**80.** The assay according to any of claims **67-79** wherein said capture line for Flt3-L comprises supported antibodies capable of binding Flt3-L bound detector reagents.

**81.** The assay according to any of claims **67-80** wherein said capture line for Rap1-GTP comprises supported antibodies capable of binding Rap1-GTP bound detector reagents.

**82.** The assay according to any of claims **67-81** further comprising non-specific IgG antibodies and/or non-specific GST fusion proteins to establish and/or increase specificity of the assay.

**83.** A kit for diagnosing early sepsis in a patient with an infection in early sepsis or an infection likely to produce sepsis comprising:

**84.** A Modular Multiplex G-Trap assay kit for measuring Rap1, Rac1 and optionally, other GTPase targets in a sample comprising reagents for up to 96 assays/target and up to 384 assays for 4 targets in multiplex format comprising:

- 1) 1-4 sets of 96 assays with red color-coded beads, each functionalized with effector proteins for a desired GTPase target;
- 2) RIPA buffer, buffer bottle; PMSF; and protease inhibitors;
- 3) Rinse and flow buffer, buffer bottle;
- 4) Optional primary antibodies (these may be user provided);
- 5) Optional secondary antibody conjugated (may be user provided);
- 6) GST-GFP for troubleshooting, 1 mL vial; and
- 7) Manual, user guide with detailed protocols and troubleshooting suggestions.

**85.** A lateral flow assay kit comprising:

- 1) A lateral flow cassette with single or multiple lanes for determining user specified analytes Flt3-L, Rap1-GTP and optionally, Rac1-GTP;
- 2) Bioconjugate markers comprising reporters (e.g., color beads, quantum dots, colloidal gel) functionalized with effector proteins or antibodies, for each of the analytes to be measured;
- 3) Lateral Flow cassettes available for qualitative analysis (color readout) or quantitative analysis (fluorescence readout);
- 4) Working Buffer reagents;
- 5) Instructions for using the assay; and
- 6) A graph setting forth standards for correlating an observed pattern of analyte captured at analyte capture lines in the serial barrier zones of said lateral flow cassettes, to analyte concentration in the test sample.

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